

WE CLAIM:

1. A protease variant comprising a substitution made at one or more of positions in a precursor protease corresponding to K170D, Y171Q and/or S173D of *Bacillus amyloliquefaciens* subtilisin.

2. The protease variant according to claim 1, further comprising a substitution at one or more positions in a precursor protease equivalent to those selected from the group consisting of N76D, S103A, V104I, G159D, V68A, T213R, A232V, Q236H, Q245R, and T260A.

3. The protease variant according to claim 2 which is derived from a *Bacillus* subtilisin.

4. The protease variant according to claim 3 which is derived from *Bacillus lentus* subtilisin or *Bacillus amyloliquefaciens* subtilisin.

5. A DNA encoding a protease variant of claim 1.

6. An expression vector encoding the DNA of claim 5.

7. A host cell transformed with the expression vector of claim 6.

8. A cleaning composition comprising the protease variant of claim 1.

9. An animal feed comprising the protease variant of claim 1.

10. A composition for treating a textile comprising the protease variant of claim 1.

11. A protease variant according to claim 1, comprising combined substitution sets selected from the group consisting of positions corresponding to K170D/Y171Q/S173D; N76D/S103A/V104I/G159D/ K170D/Y171Q/S173D;

V68A/N76D/S103A/V104I/G159D/ K170D/Y171Q/S173D/Q236H;

V68A/N76D/S103A/V104I/G159D/ K170D/Y171Q/S173D/Q236H/Q245R;

V68A/N76D/S103A/V104I/G159D/K170D/Y171Q/S173D/

A232V/Q236H/Q245R; and

- 5 V68A/N76D/S103A/V104I/G159D/K170D/Y171Q/S173D/T213R/A232V/Q236H/
Q245R/T260A of *Bacillus amyloliquefaciens* subtilisin.

12. A method for determining T-cell epitopes in humans comprising the steps
of:

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(a) obtaining from a single blood source a solution of dendritic cells and a
solution of naïve CD4+ and/or CD8+ T-cells;

(b) promoting differentiation in said solution of dendritic cells;

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(c) combining said solution of differentiated dendritic cells and said naïve
CD4+ and/or CD8+ T-cells with a peptide of interest;

(d) measuring the production of antibodies in said step (c).

of:

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(a) identifying a T-cell epitope in said protein;

(b) modifying said protein to neutralize said T-cell epitope.

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14. The method according to claim 13, wherein said epitope is modified by:

(a) substituting the amino acid sequence of the epitope with an analogous
sequence from a human homolog to the protein of interest;

(b) substituting the amino acid sequence of the epitope with an analogous
sequence from a non-human homolog to the protein of interest, which analogous
sequence produces a lesser allergenic response from T-cells than that of the protein
of interest; or

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(c) substituting the amino acid sequence of the epitope with a sequence
which substantially mimics the major tertiary structure attributes of the epitope, but
which produces a lesser allergenic response from T-cells than that of the protein of
interest.

V C1 15. A protein having reduced allergenicity made by the method according to claim 14.

5 16. A protein having reduced allergenicity, wherein said protein comprises a modification comprising the substitution or deletion of amino acid residues which are identified as within a T-cell epitope according to the assay provided in claim 13.

add C2
add 32
add E3